

REMARKS

I. Clarification and Request for Refund

The Examiner asserted in the Office Action mailed November 12, 2004, that the finality of the last action was withdrawn pursuant to 37 C.F.R. §1.114 (see Office Action at page 2). In fact, the finality of the last action was withdrawn pursuant to a **Petition** under 37 C.F.R. §1.181 filed on June 8, 2004, which was granted on October 12, 2004 by Special Program Examiner Julie Burke. The Applicants respectfully request that the Amendment and Response dated October 8, 2004 be treated as a response to a non-final action as previously requested (page 5 of said response); and request a refund of fees paid under 37 C.F.R. §1.26 in association with the conditional Request for Continued Examination under 37 C.F.R. §1.114 filed on October 8, 2004. Please refund the fee of \$790.00 paid for the Request for Continued Examination to Deposit Account No. 13-2855.

II. Remarks and Amendment

The Examiner indicated that the Applicant's previous arguments and remarks with respect to claims 7-24 and 31 have been considered moot in view of the new grounds of rejection.

Claims 7-24 and 31 are currently pending, remain rejected, and are currently under examination. Claim 14 is amended herein. Support for the amendment to claim 14 is found throughout the specification. Accordingly, the amendments do not include new matter. The Applicants do not intend with these or any other amendments to abandon the subject matter of claims previously presented, and reserve the right to pursue such subject matter in duly filed continuing patent applications.

This response is timely filed.

III. Patentability Arguments

Reconsideration and withdrawal of the rejections is solicited for the reasons set out below.

A. The Anticipation Rejection of Claims 7-12, 14-18, 20-24, and 31 under 35 U.S.C. § 102(b), May Properly Be Withdrawn.

The Examiner rejected claims 7-12, 14-18, 20-24, and 31 under 35 U.S.C. §102(b) for anticipation by Kooistra et al., *J. Bacteriol.* 126:31-37, 1976 (hereinafter “Kooistra”), because Kooistra assertedly discloses the instantly claimed invention directed to an attenuated *Pasteurellaceae* bacteria comprising a functional mutation in an *atpG* gene, wherein the functional mutation attenuates the bacteria. Office Action at pages 9-10. The Applicants respectfully traverse the rejection.

In claim 7, the Applicants are claiming an attenuated *Pasteurellaceae* bacteria comprising a mutation in the nucleotide sequence that encodes an **atpG** polypeptide, i.e., the ATP synthase F1 gamma chain (*atpG*) polypeptide. ATP synthase is an enzyme complex with multiple protein subunits. Kooistra discloses eight different mutations in *Haemophilus influenzae* leading to deficiency in ATP-dependent nuclease. Kooistra does not disclose mutations to *atpG* or mutations to specific genes or polypeptides in the ATP synthase complex. Kooistra does not disclose an attenuated *Pasteurellaceae* bacteria comprising a mutation in a nucleotide sequence that encodes an *atpG* polypeptide comprising an amino acid sequence at least 70% identical to the *atpG* amino acid sequence of SEQ ID NO: 4, said mutation resulting in decreased *atpG* biological activity, wherein the decreased *atpG* biological activity attenuates the *Pasteurellaceae* bacteria. Moreover, Kooistra never even mentions *atpG*. Kooistra does not disclose attenuation of bacteria or site-directed mutagenesis of particular bacterial genes in order to attenuate bacteria. Kooistra only discusses *Add⁻* mutations and the resultant decrease in DNA-dependent ATPase activity from the *Add⁻* mutations. The Applicants are claiming attenuated *Pasteurellaceae* bacteria comprising mutations in the nucleotide sequence that encodes an **atpG** polypeptide, **not** an *Add⁻* polypeptide.

The Examiner also alleged that “a non-functional ATPase indicates the presence of a functional mutation in the *atpG*,” (Office Action at page 10) but the Examiner does not provide evidence for such a conclusory statement. Even if Kooistra discloses *Haemophilus influenzae* mutants deficient in ATP-dependent nuclease activity, Kooistra never discloses an attenuated *Pasteurellaceae* bacteria comprising a mutation in a nucleotide sequence that encodes an *atpG* polypeptide comprising an amino acid sequence at least 70% identical to the *atpG* amino acid sequence of SEQ ID NO:4. The present invention relates to

the identification of genes responsible for virulence in *Pasteurellaceae* bacteria. Kooistra has not taught that atpG is responsible for virulence in *Haemophilus influenzae*.

For all the foregoing reasons, Kooistra has not taught what is claimed in the present invention and Kooistra cannot possibly anticipate the claimed invention. Thus, the rejection of claims 7-12, 14-18, 20-24, and 31 under 35 U.S.C. §102(b) for anticipation by Kooistra is now rendered moot and should be withdrawn.

B. The Written Description Rejection of Claims 7-24 and 31 under 35 U.S.C. §112, First Paragraph, May Properly Be Withdrawn.

The rejection of claims 7-24 and 31 under 35 U.S.C. §112, first paragraph, is maintained for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor, at the time the application was filed, had possession of the invention. Office Action at pages 2-6. The Examiner alleges that the specification does not provide adequate written description to support claims to bacteria that comprise either species homologs of SEQ ID NO: 4 or any mutation resulting in decreased activity of the protein. The Applicants respectfully disagree.

The Examiner alleged that the Applicants have provided only two sequences representing homologues to the polynucleotide and the sequences comprise numerous variations in both the polynucleotide and the encoded polypeptide sequences. Further, the Examiner alleged that the Applicants have not provided any common structural core which one skilled in the art could use to identify a genus of polynucleotides in the attenuated bacteria of the Family *Pasteurellaceae* and alleges that the Applicants are claiming polynucleotide homologs only by their functionality or lack thereof. Office Action at page 3. As the Patent Office recognizes, the issue of written description involves the question of whether the specification shows that the Applicants had possession of the claimed invention. The Applicants are claiming bacteria and they are claiming said bacteria comprising a mutation in a nucleotide sequence with a specific structure: function relationship in the claims. The Applicants are not claiming polynucleotide sequences per se.

Claim 7 recites “an attenuated *Pasteurellaceae* bacteria comprising a mutation in a nucleotide sequence that encodes an atpG polypeptide comprising an amino acid sequence at least 70% identical to the atpG amino acid sequence of SEQ ID NO:4, said

mutation resulting in decreased atpG biological activity, wherein the decreased atpG biological activity attenuates the *Pasteurellaceae* bacteria.” The Applicants are not claiming polynucleotide homologs and they are not claiming such homologs only by their function. The claims are not drawn to polynucleotides but rather, an organism (bacteria) comprising a genetic modification at a particular locus (gene), providing a specified change of phenotype. Thus, case law pertaining to DNA inventions is clearly distinguishable from this invention. Moreover, insofar as one element of the claims involves a sequence limitation, there is clearly written descriptive support in the specification for gene sequences that possess 70% identity to the claimed sequences (see specification at page 19, lines 3-19). There is also support in the specification for a defined function, atpG biological activity, and it is found in Example 10 (see specification at pages 47-51) where the specification describes how atpG functions in ATP synthase, e.g., by the production of ATP or in the transport of protons by hydrolyzing ATP. The specification also describes how the loss of atpG biological activity attenuates the bacteria (see Examples 3, 9, and 11). The specification also provides two species of the claimed genus of the atpG polypeptide in *P. multocida* (e.g., SEQ ID NO: 4), the base sequence information of the atpG polypeptide in *A. pleuropneumoniae* (e.g., SEQ ID NO: 133).

Contrary to what the Examiner alleges in citing *Amgen v. Chuzai*, 18 U.S.P.Q.2d 1016 (CAFC 1991) and *University of California v. Eli Lilly and Co.*, 43 U.S.P.Q.2d 1398 (1997) (Office Action at pp. 3-5), the presently claimed invention is not defining the amino acid sequence in the claimed bacteria only by its function. Instead, claim 7 defines the amino acid sequence in the claimed bacteria by a combination of structural (at least 70% identical to the atpG amino acid sequence of SEQ ID NO:4) and functional properties (decreased atpG biological activity, wherein the decreased atpG biological activity attenuates the *Pasteurellaceae* bacteria). The structural feature common to all members of the claimed genus is “at least 70% identity to the amino acid sequence of SEQ ID NO: 4,” which is provided in the specification. Thus, the claimed invention meets the requirements of “Product by Function” set forth in Example 14 of the “Revised Interim Written Description Guidelines” published by the Patent Office, because the encoded polypeptides and the variants thereof in the claimed bacteria are described as possessing at least 70% identity to the claimed sequences wherein said “mutation results in decreased atpG biological activity, wherein the decreased atpG biological activity attenuates the *Pasteurellaceae* bacteria,” thus providing a structure: function attribution. The Applicants have disclosed two species of the

claimed atpG genus and members of the claimed genus can be identified by these common attributes.

In addition, the specification provides the identification of atpG genes by Southern hybridization in several species of the claimed genus of *Pasteurellaceae* bacteria, e.g., *Pasteurella haemolytica*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, and *Haemophilus somnus* (see Example 5, pages 39-41). One of skill in the art can readily isolate and sequence atpG genes from bacterial species that can be identified and mapped using Southern hybridization and related techniques, and target said genes using site-directed mutagenesis or other methods. The use of a sequence similarity limitation (at least 70% identity) harmonizes with the ability to identify other atpG genes by hybridization because hybridization occurs between similar (complementary) sequences. Thus, the invention involves the identification of the atpG gene as being important to bacterial virulence, and shows that the disruption of the atpG gene attenuates the *Pasteurellaceae* bacteria. The hybridization experiment shows that the Applicants were in possession of the claimed invention, currently defined in part by sequence similarity to sequences provided in the application. For example, there is ample precedent for defining a genus by sequence similarity. The hypothetical example (Example 14) in the Guidelines teaches only one species, yet allows a claim to sequences having 95% identity. The Applicants submit that one of skill in the art is in possession of the attributes and features of each species in the claimed genus. The Applicants should be permitted to claim as broadly as the art allows and the specification describes and enables.

The Applicants have demonstrated that *Pasteurellaceae* bacteria with disruptions in the open reading frame of the atpG gene are attenuated and are capable of providing protective immunity against wild-type bacteria of the same strain. The claims are directed, e.g., to an attenuated bacteria comprising an atpG mutation. As described in the specification (see Examples at pages 30-53), it is not necessary to know the sequence of an atpG homolog to make and use the claimed invention. Signature-tagged mutagenesis was used to identify bacterial genes required for virulence. Bacterial strains, each with a random mutation in the genome, were produced using transposon integration with each insertional mutation carrying a different DNA signature tag allowing mutants to be differentiated from each other. The tags comprised 40 bp variable central regions flanked by invariant "arms" of 20 bp which allowed the central portions to be co-amplified by PCR. Tagged mutant strains were assembled in microtiter dishes, then combined to form the "inoculum pool" for infection

studies. At an appropriate time after inoculation, bacteria were isolated from the animal and pooled to form the "recovered pool." The tags in the recovered pool and the tags in the inoculum pool were separately amplified, labeled, and then used to probe filters arrayed with all of the different tags representing the mutants in the inoculum. Mutant strains with attenuated virulence were those which could not be recovered from the infected animal, *i.e.*, strains with tags that give hybridization signals when probed with tags from the inoculum pool but not when probed with tags from the recovered pool. The nucleotide sequence of the open reading frame disrupted by the transposon insertion was determined by sequencing both strands and an encoded amino acid sequence was deduced. Consequently, it was not necessary to know the complete sequence of the *Pasteurella multocida* atpG gene to construct the mutant in Example 1 or to demonstrate that the mutant is attenuated in Example 2. The same is true for other *Pasteurellaceae* family members, as demonstrated in *Actinobacillus pleuropneumoniae* in Example 5, and there is nothing of record to suggest that this would not be true for any *Pasteurellaceae* family members. Thus, the specification provides ample description of structural and functional characteristics sufficient to identify members of the claimed genus. Thus, the Applicants have described the organism, the gene, the type of change to make (mutation), and the phenotype (attenuated) to select. These facts clearly distinguish this invention from inventions involving litigated claims to isolated DNA molecules of alleged function, cited in the Office Action.

The Applicants also maintain their position that they do not need to specifically point out where the mutation is located within the atpG sequence, as long as the mutation disrupts the expression or function of the encoded atpG polypeptide. As submitted in a previous response, in claims to transgenic animals comprising a "knockout" of a particular endogenous gene, the claims are not limited to the specific site of disruption within the sequence or the sequence identification number disclosed, see e.g., U.S. Patent Nos.: 5,714,667; 5,777,195; 6,087,555; and 6,100,445. The presently claimed attenuated bacteria are analogous to knockout animals having a particular phenotype because the patents at issue do not describe the sequence of every homolog of the gene of every mouse within the scope of the issued claims. The claimed genus of attenuated bacteria recite a combination of functional (decreased atpG activity) and structural (polypeptide comprising an amino acid sequence at least 70% identical to the atpG amino acid sequence of SEQ ID NO:4) properties. The claimed bacteria comprise a mutation in a polynucleotide encoding atpG, which results in the disrupted function of atpG activity, resulting in attenuation. The specification provides

adequate written descriptive support for mutating an *atpG* polypeptide to result in attenuation of *Pasteurellaceae* bacteria. In fact, the specification provides two working examples (Examples 9 and 11) of attenuated *Pasteurellaceae* bacteria, *Pasteurella multocida* and *Actinobacillus pleuropneumoniae*, comprising mutations in the open reading frame of the *atpG* gene (SEQ ID NO: 3 in *Pasteurella multocida* and SEQ ID NO: 132 in *Actinobacillus pleuropneumoniae*), which demonstrated decreased virulence in animals. Thus, the specification describes the claimed invention.

For these reasons and the reasons of record, the Applicants respectfully request reconsideration and withdrawal of the rejection of claims 7-24 and 31 under 35 U.S.C. §112, first paragraph, for lack of written description.

C. The Enablement Rejection of Claims 7-24 and 31 under 35 U.S.C. §112, First Paragraph, May Properly Be Withdrawn.

The Examiner rejected claims 7-24 and 31 under 35 U.S.C. §112, first paragraph, for allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art that the inventor to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Office Action at pages 6-9. The Examiner alleges that the Applicants have not provide sufficient disclosure to enable one of skill in the art to make any mutants of any *Pasteurellaceae* *atpG* polynucleotide, or a bacterium comprising such, wherein the mutation results in a decreased activity of a gene product because the Applicants have not provided sufficient information for one skilled in the art to make or use the claimed polynucleotides without undue experimentation. The Examiner also alleged that although the claims read on any mutation to SEQ ID NO: 4, and to homologs thereto that have the effect of decreasing the activity of the gene product, the Applicants have neither provided any direction or guidance, nor any working examples in the specification as to any potential mutations of SEQ ID NO: 4 that would satisfy the limitations of the claims. Numerous substitutions, deletions, and insertions may be made in the polynucleotide sequence, but would require undue experimentation because the Applicants have claimed broadly and not provided sufficient guidance. The Applicants respectfully traverse the rejection.

As the Patent Office recognizes, the issue of enablement involves the question of whether an application enables one of ordinary skill in the art to make and to use the

claimed invention. The application teaches a bacteria comprising a mutation in a nucleotide sequence that encodes an atpG polypeptide comprising an amino acid sequence at least 70% identical to the atpG amino acid sequence of SEQ ID NO: 4, and teaches that the mutation results in decreased atpG biological activity, wherein the decreased atpG biological activity attenuates the *Pasteurellaceae* bacteria. The Applicants are not claiming polynucleotide homologs and they are not claiming such homologs only by their function. Rather, the claims are drawn to an organism (bacteria) comprising a genetic modification at a particular locus (gene), providing a specified change of phenotype. Given the base sequence information of the atpG polypeptide in *P. multocida* (e.g., SEQ ID NO: 4), the base sequence information of the atpG polypeptide in *A. pleuropneumoniae* (e.g., SEQ ID NO: 133), the specifically identified function of the encoded polypeptide molecule ("atpG biological activity"), and the well-known techniques for isolating atpG genes from a variety of *Pasteurellaceae* bacteria species (see Example 5 in the specification at pages 39-41), one skilled in the art clearly would be able to identify, and make, the claimed *Pasteurellaceae* bacteria comprising variants of the atpG polypeptide using no more than routine experimentation.

In the instant case, the quantity of experimentation would be insignificant because the Applicants have taught the reference polynucleotide sequence in SEQ ID NO: 3 and the reference amino acid sequence in SEQ ID NO: 4, and the activity for which to assay, atpG biological activity. The specification 1) teaches the properties of variants of the polynucleotide and polypeptide virulence products (see specification at page 19, line 3, through page 21, line 37) and 2) provides the identification of atpG genes by Southern hybridization in several species of the claimed genus of *Pasteurellaceae* bacteria, e.g., *Pasteurella haemolytica*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, and *Haemophilus somnus* (see Example 5, pages 39-41). One of skill in the art can readily isolate and sequence atpG genes from bacterial species that can be identified and mapped using Southern hybridization and related techniques, and target said genes using site-directed mutagenesis or other methods. Thus, one of skill in the art would be able to identify and make variants of these sequences using well known techniques without undue experimentation. The use of a sequence similarity limitation (at least 70% identity) harmonizes with the ability to identify other atpG genes by hybridization because hybridization occurs between similar (complementary) sequences. Thus, the invention involves the identification of the atpG gene as being important to bacterial virulence, and shows that the disruption of the atpG gene attenuates the *Pasteurellaceae* bacteria. Suitable

assays for identifying atpG activity were well known to one of skill in the art prior to the filing date of the instant application (see Example 10). This guidance, coupled with the teachings in the specification for making bacteria with mutant sequences and screening for attenuation (see pages 31-53) will allow one of skill in the art to make and identify bacteria within the scope of the claimed invention.

Consistent with this guidance, the Applicants have disclosed an additional species, for example, the polynucleotide sequence set forth in SEQ ID NO: 132, which encodes a polypeptide having the amino acid sequence set forth in SEQ ID NO: 133, which shares 74% amino acid identity and 89% amino acid positivity with SEQ ID NO: 4 (see Exhibit A). Another factor weighing in favor of concluding that the claims are enabled is the relative skill of those in the art, which is generally recognized as being quite high. Although the Examiner has alleged that “the sequences are set out [in the application], and it is left to those in the art to run comparisons to determine what the similarities are among them are. . .” (Office Action at page 8), the Applicants submit that sequence comparisons are routine in the art and the level of skill is quite high. Furthermore, the Applicants do not need to specifically point out where the mutation is located in the sequence and thus the Examiner’s reliance upon Bowie et al., *Science* 247:1306-1310, 1990 (Office Action at page 8) is irrelevant. Indeed, in claims to more complicated *transgenic animals* comprising a “knockout” of a particular endogenous gene, the Patent Office does not require that claims be limited to the specific disruption or the particular sequence identification number disclosed, see e.g., U.S. Patent Nos. 5,714,667; 5,777,195; 6,087,555; and 6,100,445. The presently claimed attenuated bacteria are analogous to knockout animals having a particular phenotype. The rejection does not put forth any reasoning as to why a claim directed to a bacteria lacking expression of a particular gene must be limited to the particular sequence of the mutation, whereas a claim directed to an animal lacking expression of a particular gene does not. Bacteria have a simpler and smaller genome and are easier to manipulate than animals, and the enablement requirements for an attenuated bacteria (defined by gene disruption) should not be more stringent than the enablement requirements for transgenic “knockout” mice.

The Examiner also rejected claims 7-24 and 31 for allegedly not describing how a mutation in the protein coding region of a gene can result in decreased expression of the gene product nor identified any such mutation, and it is unclear how such a mutation can affect gene expression because mutations affecting gene expression are generally in the non-coding region. In response, the Applicants respectfully traverse the rejection and submit that

the Examiner's rejection is misdirected as it relates to claims 7-13, 15-24, and 31. Claims 7-13, 15-24, and 31 do not claim that a mutation in the protein coding region results in decreased expression of an atpG gene transcript. Rather these claims are drawn to bacteria with a mutation in the protein coding region which results in decreased atpG biological activity, an inactive atpG polypeptide, or decreased atpG polypeptide expression. The amendment to claim 14 herein renders the rejection moot. As the Examiner acknowledges, one of skill in the art knows that mutations to the coding region of a gene can result in decreased biological activity or decreased protein expression. Thus, this rejection has been overcome-in-part and rendered moot-in-part.

For all of the foregoing reasons, the Applicants respectfully request reconsideration and withdrawal of the rejection of claims 7-24 and 31 under 35 U.S.C. §112, first paragraph, for lack of enablement.

IV. Conclusion

In view of the arguments/remarks made herein, the Applicants respectfully submit that claims 7-24 and 31 are in condition for allowance and respectfully request expedited notification of same. Should the Examiner have any questions, she is welcomed to contact the undersigned at the telephone number below.

Respectfully submitted,

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Registration No. 53,066

February 14, 2005



Blast 2 Sequences results

PubMed

Entrez

BLAST

OMIM

Taxonomy

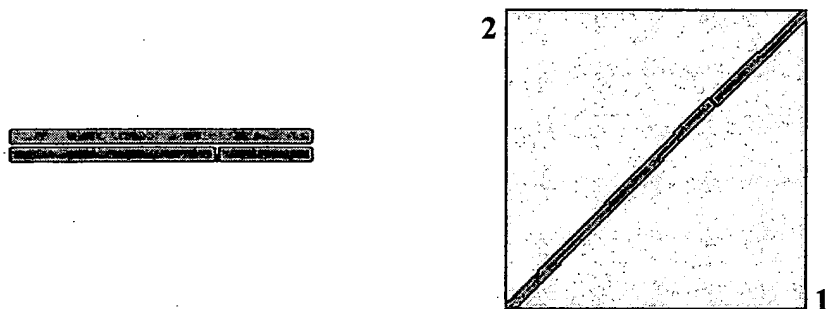
Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.10 [Oct-19-2004]

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Sequence 2 lcl|seq_2 Length 288 (1 .. 288)



NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

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Identities = 215/289 (74%), Positives = 260/289 (89%), Gaps = 1/289 (0%)

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Gapped

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Number of Hits to DB: 717

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Length adjustment: 129

Effective length of query: 160

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X2: 129 (49.7 bits)

X3: 129 (49.7 bits)

S1: 42 (22.0 bits)

S2: 50 (23.9 bits)